Detection of postharvest pathogenic fungi by RNA markers in high-throughput platform on streptavidin plate

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Abstract

Postharvest pathogenic fungi cause rots and major losses in harvested crops worldwide. Therefore, advanced disease detection and decay prevention in crops are essential to minimize these rots. In this study, oligonucleotides-based detection was integrated into an adapted commercial well-plate reader to enable a high-throughput biosensor monitoring system for the detection of RNA-markers of quiescent fungi in harvested crops. The streptavidin commercial 96-well plates were functionalized with complementary strands to the target RNA-markers of quiescent fungal-pathogens *Alternaria alternata* and *Botrytis cinerea*. After exposure to the target sample, the target RNA sequence binds to the immobilized surface DNA strand. Then, a reporter DNA strand binds to the target RNA. The reporter DNA that is linked to the fluorophore Texas-Red produces a light signal, which is detected by the plate reader. Firstly, the assay functionality was optimized with the surface DNA strand and reporter DNA strand concentrations of 100 nM and 250 nM, respectively. Then, the specificity of the platform was examined against several non-specific target DNA markers from different pathogenic fungi. The light intensity signals were significantly higher by 3.6- and 4.9-fold as compared to the control in the markers of *Botrytis cinerea* and *Alternaria alternata*, respectively. The biosensor demonstrated highly sensitive detection for both *Botrytis cinerea* (LOD = 0.657 nM RNA), and *Alternaria alternata* (LOD = 0.533 nM RNA), which was validated with biological samples. The obtained valuable information from this platform allows the early detection of RNA-markers of quiescent pathogenic fungi in agricultural produce, which will provide a smart and data-based decision-making tool to reduce postharvest losses.